CORRECT VERSION OF THE CLAIMS

1. (Twice Amended) A reversible physiological process for temporal separation of oxygen evolution to avoid deactivation of hydrogenase in the presence of oxygen and sustain photosynthetic hydrogen production in cells of an algal microganism, comprising:

(a) growing a culture of cells of algal microorganism in a medium under illuminated conditions to accumulate an endogenous substrate;

(b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium until the culture of cells of algal microorganism becomes anaerobic and sealing the culture from atmospheric oxygen;

(c) measuring the rate of cellular respiration of a sample of cells of the algal microorganism from step (b) in the dark with an O_2 electrode;

(d) incubating a sample of the algal microorganism from step (c) in light of saturating intensity of yellow actinic excitation, and measuring the light-saturated rate of O_2 evolution with an O_2 electrode;

(e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algal microorganism of step (b) so that it is equal to or less than a rate of cellular respiration; and

(f) collecting an evolved gas that includes hydrogen.

REMARKS

Applicants acknowledge with appreciation the courtesy of Examiner Afremova in discussing the 10/2/02 Proposed Amendment Under §1.116 during a telephone conversation on October 22, 2002. During the interview it was suggested that more detail be provided regarding: 1) the manner of measuring cell respiration in step c); 2) measuring the O_2 evolution in step d); and 3) controlling O_2 production so that it is equal to or less than the rate of cellular respiration in step e).

Applicants have revised claim 1 consistent with said suggestions – and in view thereof, it is believed that the interview has materially advanced the prosecution of this application.

The Official Action and the cited references have again been carefully reviewed. The review shows that the claims, especially as amended, recite patentable subject matter and should be allowed. Reconsideration and allowance are therefore respectfully requested.

Prior to contending with the grounds upon which the rejections have been based, a brief summarization of the prior art background along with applicants' inventive contribution will be provided to facilitate easier grasp of applicants' process of continuously producing hydrogen by inducing reversible hydrogenase in a manner that provides activity of photosynthesis from a light saturated rate of oxygen production equal to or less than a rate of cellular respiration of an algal microorganism.

In the prior art where it is known that algae will not produce hydrogen gas when oxygen is present because the hydrogenase enzyme that releases hydrogen is not synthesized and is not stable when oxygen is present, and where the normal plant/algal photosynthetic process splits water and produces oxygen as a by-product, and wherein to get algae to induce the hydrogenase enzyme it has been necessary to use physical (i.e., inert gas

bubbling) or chemical (addition of strong reducing agents or biochemical, oxygen-scrubbing systems) means to get rid of the oxygen, applicants have discovered a metabolic switch, whereby removing sulfate from the medium of healthy growing algae rapidly decreases the innate ability of the algae to split water and produce oxygen to about 10% of their normal ability over a 15 to 30 hour period of time. In this sulfate removal process, applicants have further discovered that algal respiration can take up oxygen at about the level or a little greater (rate)than the cells can produce oxygen (at the lower level of production ability) under sulfur-deprived conditions, and that the culture under these conditions will metabolize all the remaining oxygen in the culture medium, and the system will become anaerobic. Hydrogen is produced under these conditions because the hydrogenase enzyme can be induced by the cells and it is stable in the absence of oxygen.

The reversible physiological process to avoid deactivation of hydrogenase in the presence of oxygen and sustained photosynthetic hydrogen production is accomplished by:

(a) growing a culture of cells of algal microorganism in a medium under illuminated conditions to accumulate an endogenous substrate;

(b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium until the culture of cells of algal microorganism becomes anaerobic and sealing the culture from atmospheric oxygen;

(c) measuring the rate of cellular respiration of a sample of cells of the algal microorganism from step (b) in the dark with an O_2 electrode;

(d) incubating a sample of the algal microorganism from step (c) in light of saturating intensity of yellow actinic excitation, and measuring the light-saturated rate of O_2 evolution with an O_2 electrode;

(e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algal microorganism of step (b) so that it is equal to or less than a rate of cellular respiration; and

(f) collecting an evolved gas that includes hydrogen.

Claims 1-3, 5-8 and 10 were rejected as being anticipated by Greenbaum '211 under 35 USC §102(b).

Applicants respectfully traverse this rejection and request reconsideration for the reasons which follow.

Greenbaum '211 disclose a method of producing H_2 and O_2 by use of algae in light comprising:

1) subjecting algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 to produce H_2 and O_2 by the action of the light-stimulated algae in splitting water molecules during a first period of time of sufficient duration to produce a physiological stress on said algae;

2) culturing said algae in culture medium in an aerobic atmosphere during a second period of time sufficient to remove said physiological stress; and

3) subjecting the algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 during a third period of time at an enhanced rate of production of H_2 and O_2 compared to that occurring during the first time period of step (1).

Most notably, Greenbaum '211 lacks applicants' step (b) of depleting sulfur until the culture becomes anaerobic. This alone incapacitates Greenbaum of anticipating applicants' process.

Further, Greenbaum '211 also lacks applicants' steps (c),(d) and (e) which includes inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production equal to or less than the rate of cellular respiration. Accordingly, this compounds the initial error of applying Greenbaum.

Withdrawal of the rejection is respectfully requested.

Claims 1-3, 5-8 and 10 were rejected as being unpatentable over Greenbaum '211 taken with Weetall '076, Wykoff et al. and Melis.

Applicants respectfully traverse this rejection and request reconsideration for the following reasons.

Greenbaum '211 has been discussed at length above; however, it is worthwhile to reiterate that Greenbaum '211 lacks applicants' step of depleting the sulfur nutrient until the culture becomes anaerobic as well as the steps (c), (d) and (e), which includes inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production equal to or less than the rate of cellular respiration.

The aforementioned deficiencies in Greenbaum '211 are not provided in the secondary references of Weetall, Wykoff et al. and Melis.

This is so because Weetall only disclose a method of continuous photometabolic production of a useful product which comprises the steps of immobilizing whole cells of a photometabolically active organism on a medium to form a stabilized composite, supportably placing the composite within a reactor having at least one light transmitting wall, and, in the presence of light being transmitted through the wall, continuously passing into the reactor a substance capable of being photometabolized by the cells under conditions sufficient to assure the production of the useful product.

Although blue-green algae may be used in the biophotolysis of water by oxidizing the water and reducing NADP to NADPH, any combination of Weetall with Greenbaum '211 would still be deficient with regard to providing applicants' steps (b) and (d), which are the steps of depletion of the sulfur nutrient and incubating the culture in light to induce reversible hydrogenase to provide activity of photosynthesis from a light saturated rate of oxygen production, equal to or less than the rate of cellular respiration.

The deficiencies of Greenbaum '211 and Weetall are not compensated for by any teachings in the secondary references of Wykoff et al., and Melis Anastasios.

Wykoff et al. merely disclose the extent to which the light-saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation. The publication makes no reference to or acknowledgement of the problem of not being able to sustain hydrogen production by virtue of the deactivation of hydrogenase in the presence of oxygen during photosynthetic hydrogen production. Neither does Wykoff et al. provide any solution to this problem.

Melis is applicants' own publication published after the present application was filed. Applicants have incorporated herewith, an Affidavit Under 37 CFR §1.131 swearing back of this reference. Accordingly, this reference is not available against the present invention.

Even if the teachings in the secondary reference of Wykoff et al. were combined with the teachings of Greenbaum and Weetall, applicants' invention as presently recited would clearly not result. Neither would applicants' invention be rendered obvious because none of the references address nor resolve the problem of providing sustained production of hydrogen by avoiding deactivation of hydrogenase in presence of oxygen.

Thus, even if the Wykoff et al. teachings of the extent to which the light saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation were substituted into the processes of the primary references, such a substitution would clearly not provide a skilled person in the art with means for sustaining production of hydrogen by avoiding deactivation of hydrogenase in the presence of oxygen, as required by applicants' claims, as presently amended.

Withdrawal of the rejection is respectfully requested.

Note is taken of the rejections of claims 1-3, 5-8 and 10 under the second paragraph of 35 USC 112 on grounds of indefiniteness; however, in view of the amendments made to these claims, the rejections are no longer applicable.

Note is taken of the indication that the Affidavit filed under 37 CFR §1.131 was not signed by all of the inventors; however, re-submitted herewith is the Affidavit signed by all of the inventors.

In view of the foregoing amendments, remarks, Affidavit under 37 CFR § 1.131, and arguments advanced, it is believed that the application is now in condition for allowance and early notification of the same is earnestly solicited.

Respectfully submitted,

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